SYMPOSIUM ON AUTOTROPHY[1]

III. Recent Developments in Photosynthesis[2]

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I. Photosynthesis and Biochemical Unity, 1930–1950

In bacterial photosynthesis[4] the reduction of carbon dioxide is obligatorily coupled with the oxidation of a hydrogen donor such as H_2, H_2S, and other simple organic compounds. Green plants require no such H-donor for the photosynthetic assimilation of CO_2; they can obtain reducing power by eliminating O_2 from H_2O. These and other comparative features of photosynthesis were incorporated into a scheme, developed largely by van Niel (94, 98) during the period 1930 to 1950, that afforded a unified concept of photosynthesis and promised a unification of mechanisms in photo- and chemoautotrophy.

The essence of van Niel's formulation, illustrated in Fig. 1, is that neither CO_2 nor the H-donor substrate (H_2A in a general formulation) participates directly in a photochemical reaction involving chlorophyll. The central event is a photolysis of water, yielding a reducing entity symbolized as (H) and an oxidizing entity called (OH). The H-donor, H_2A, is needed to dispose of (OH), whereas (H) brings about the reduction of CO_2. In this scheme the details of energy transfer and utilization are left unspecified, but quantum yield measurements with bacteria utilizing various H-donor substrates have shown that the oxidation of H_2A does not yield energy useful in photosynthesis (69, 103). Even where H_2A is an organic substrate, its role is thus held to be restricted: it is primarily an agent for removing (OH) and not a source of energy or of cell carbon. This view was strengthened when Foster (36, 37) showed that isopropanol, acting as H_2A in bacterial photosynthesis, was oxidized quantitatively to acetone: $CH_3CHOHCH_3 \rightarrow CH_3COCH_3 + 2(H)$. To be sure, van Niel (94) allowed for the possibility that an organic H-donor could yield oxidized intermediates other than CO_2 and that these could become assimilated photosynthetically. But such reactions were regarded as minor variations on the central theme of CO_2-assimilation.

Once the reduction of CO_2 and the oxidation of H_2A had been separated from the primary photochemical event, a relationship between photosynthesis and chemoautrotrophy became obvious. Identical pathways of CO_2 assimilation could be postulated for the two ways of life; in photosynthesis the mobilization of light energy involves the photo-oxidation of H_2A, whereas in chemoautotrophy energy is supplied through the aerobic oxidation of H_2A. This comparison

[1] This symposium was held at the Annual Meeting of the American Society for Microbiology, Chicago, Ill., 24 April 1961, under the sponsorship of the Division of Agricultural and Industrial Bacteriology, with Robert L. Starkey as convener.

[2] An abundance of very recent information will necessitate reappraisal of many areas in this mobile field of investigation.

[3] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

[4] The photosynthetic bacteria include the sulfur and non-sulfur purple bacteria and the green sulfur bacteria. Their taxonomy and physiology is described in part by van Niel (97) and Larsen (69).

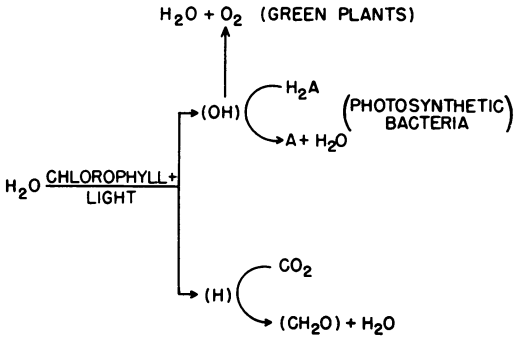

FIG. 1. *A representation of van Niel's formulation of photosynthesis of 1949 (98). Water is split photochemically into a reducing entity (H) and an oxidizing entity (OH). The former brings about reduction of CO_2 to cell materials (CH_2O); the latter is disposed of through the oxidation of a hydrogen donor, H_2A (in photosynthetic bacteria), or through the elimination of O_2 (in green plants).*

is straightforward if H_2A is inorganic. A consideration of cases in which H_2A is organic invites speculation as to the unity of autotrophy and heterotrophy.

The idea of a close relationship between photosynthesis, chemoautotrophy, and heterotrophy gained considerable force through two observations. First, a variety of photosynthetic bacteria that use organic substrates as H-donors in photosynthesis can grow equally well in darkness, deriving energy by aerobic oxidation of the same substrates (98). This capability has also been found with purple bacteria using H_2 as the substrate (96). Second, green algae, which normally exhibit "green plant" photosynthesis, can be trained by exposure to H_2 to perform a "bacterial" photosynthesis in which H_2 is the substrate and no O_2 is evolved (46). The same H_2-adapted algae can assimilate CO_2 in darkness while oxidizing H_2 with O_2 (47). Thus one organism can be made to exhibit both types of photosynthesis and also a chemoautotrophic reaction.

II. Changing Conceptions of Photosynthesis

A. Role of H-Donor Substrate

The development of current theories of photosynthesis has been reviewed recently by Stanier (82); a more condensed account will be given here.

Van Niel's formulation of photosynthesis (Fig. 1), together with arguments invoking biochemical unity, led to certain attitudes. The distinctive feature of photosynthesis is a separation of oxidizing and reducing power through a photolysis of water. The function of H_2A is primarily to reduce (OH), preventing a wasteful recombination of (H) with (OH) and leaving (H) free to reduce CO_2. The carbon of cell materials comes chiefly from the assimilation of CO_2. The pathways of CO_2 assimilation and of H_2A oxidation are essentially the same, in photosynthetic bacteria, whether the metabolism is based on photosynthesis or (in darkness) on respiration.

A variety of observations have accrued that weaken or contradict these attitudes.

As early as 1933, Gaffron observed (44, 45) in purple bacteria a photosynthetic assimilation of fatty acids with the formation of a storage product of empirical formula $(C_4H_6O_2)_n$. Small amounts of CO_2 were concomitantly fixed or released, as required to preserve an over-all oxidation-reduction balance. These observations appeared to deny the preponderance of CO_2 as a source of cell carbon and suggested a less restricted role for "H_2A" (in this case a fatty acid). But van Niel (94) interpreted Gaffron's observations as follows: the fatty acids, acting in the role of H_2A, are oxidized to the level of CO_2; the CO_2 is in turn assimilated. At the same time van Niel relaxed his position to allow the assimilation not only of CO_2 but also of organic molecules arising in the oxidation of H_2A. This more flexible position was reasserted in 1954 (99) in response to Gest's observation (51) that organic substrates could serve, in bacterial photosynthesis, in capacities other than that of H-donor. There being no incisive way to choose between Gaffron's and van Niel's interpretation, the latter prevailed by virtue of its elegance and clarity. We shall return later to a more conclusive treatment of this question, based on a modern elaboration of Gaffron's work. Meanwhile three points should be noted.

The photochemical conversion of isopropanol to acetone, celebrated as an unequivocal demonstration of the H-donor function of organic compounds in bacterial photosynthesis, has emerged as a singular reaction and has correspondingly lost significance as a model for organic substrates generally.

Glover and Kamen (52) and Ormerod (76) have shown that the photosynthetic assimilation of $C^{14}O_2$ in purple bacteria is depressed when acetate

is added to the cell suspension. To accommodate this observation to the attitude that acetate acts as H-donor for CO_2 assimilation, one must assume that CO_2 arising from the oxidation of acetate is assimilated in preference to exogenous CO_2.

Application of the principle of biochemical unity dictated that in purple bacteria the oxidation of H_2A should follow the same pathway in photosynthetic and in oxidative (aerobic) metabolism. The aerobic oxidation of organic acids in purple bacteria proceeds via the tricarboxylic acid cycle (28, 29). In contrast, the metabolism of these acids in illuminated, anaerobic cells, as revealed in experiments using fluoracetate as a metabolic inhibitor, is strikingly different; the tricarboxylic acid cycle appears not to be a major pathway (29).

The foregoing considerations are in no instance conclusive. In recent years, however, two major developments have forced a revision of the now classical scheme advanced by van Niel. One of these was the discovery and partial elucidation of photosynthetic phosphorylation. The other was the resumption and elaboration, by Doudoroff, Stanier and collaborators, of Gaffron's early experiments concerning the photometabolism of organic acids in purple bacteria. The storage product $(C_4H_6O_2)_n$ detected by Gaffron (44, 45) was again brought to light and identified by Doudoroff and Stanier (25) as poly-β-hydroxybutyric acid. Stanier et al. (83) studied the photometabolism of organic acids in non-sulfur purple bacteria that were nitrogen-starved and hence unable to synthesize proteins. They found that the principal assimilation products are poly-β-hydroxybutyric acid and a polysaccharide resembling glycogen. The former is the main product of acetate and butyrate metabolism; the latter predominates when propionate, pyruvate, dicarboxylic acids, or $CO_2 + H_2$ are fed to the cells. C^{14}-labeled acetate and butyrate were found (25) to be converted almost quantitatively, with little loss of specific activity, to poly-β-hydroxybutyric acid. In the reductive polymerization

$$\text{Acetate} \rightarrow \text{poly-}\beta\text{-hydroxybutyric acid}$$

a small share of the acetate is oxidized to CO_2 to provide the necessary reducing power. In the presence of H_2 this oxidation is avoided, the reductive polymerization proceeds more rapidly, and the yield of poly-β-hydroxybutyric acid from acetate is essentially quantitative. Finally it was shown that β-hydroxybutyric acid is polymerized in illuminated purple bacteria, with β-hydroxybutyryl-coenzyme A as an intermediate (73, 82). Moreover, a light-dependent polymerization of glucose to starch has been observed in leaves (71).

These observations forced a new attitude toward the role of "H_2A" in photosynthesis. Where H_2A is inorganic and CO_2 is the sole source of carbon, the function of H_2A is surely that of H-donor; cell materials are made from CO_2 via the pentose cycle (18, 84). But organic species of H_2A are incorporated into cell materials by a variety of assimilatory processes; often little or none of the substrate is oxidized and CO_2 plays only a minor part in preserving an over-all oxidation-reduction balance. The direct polymerizations of β-hydroxybutyric acid and glucose are of special interest. Here the photosynthetic substrate is in no sense a hydrogen donor; there is no need to invoke a separation of oxidizing and reducing power because nothing is oxidized or reduced. All that is needed for these polymerizations is energy, or, in modern currency, adenosine triphosphate (ATP).

B. Photosynthetic Phosphorylation and Generation of Reducing Power

The foregoing view of assimilatory metabolism in photosynthesis was consolidated by the prior discovery of photosynthetic phosphorylation (11, 41). In terms of a photolysis of water (Fig. 1), the recombination of (H) and (OH) is not a wasteful process; rather it represents an electron transfer mechanism that is coupled to the generation of ATP from adenosine diphosphate (ADP) and inorganic phosphate. The ATP can then drive the various assimilatory reactions. Reducing power is still needed, of course, when the source of carbon is more oxidized than the assimilation product, and the green plants must generate this reducing power from water. Let us turn now to a consideration of how photosynthetic tissues generate ATP and high-potential reducing power.

Photosynthetic phosphorylation was demonstrated convincingly in chloroplasts by Arnon, Allen, and Whatley (11) and in the chromatophores (the photosynthetically active subcellular particles) of purple bacteria by Frenkel

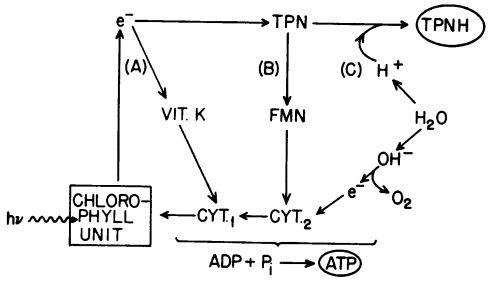

FIG. 2. *An abbreviated description of Arnon's scheme (9) for the photosynthetic generation of chemical bond energy (ATP) and reducing power (TPNH). A chlorophyll unit absorbs light and produces a high-energy electron. The electron is returned by pathways that are coupled with phosphorylation; one pathway affords the reduction of TPN together with the evolution of O_2 from water.*

(41). The attendant biochemical details are numerous and not fully elucidated; they have been reviewed extensively (48, 100) and will receive only a summary exemplification here.

The diagrams representing photophosphorylation have usually incorporated a primary photolysis of water (8, 60). However, it is possible, as we shall see, to conceive of a unit of chlorophyll, protein, lipid, and enzymes that performs the primary photochemical process without the chemical involvement of water. We may examine the outlines of photophosphorylation, then, through a model advanced by Arnon (9) that omits the symbolism of photochemical water cleavage. Arnon's model is shown, somewhat abbreviated, in Fig. 2. Chlorophyll absorbs light and consequently makes available high-energy electrons. The electrons can be returned to the chlorophyll unit via pathways (*A* and *B*) that are coupled to a reaction yielding ATP from ADP and inorganic phosphate. Through this "cyclic phosphorylation" the energy of the electrons is converted in part to chemical bond energy. Alternatively (path *C*) the electrons, together with protons from water, can reduce triphosphopyridine nucleotide (TPN) while other electrons are abstracted from OH^- ions (with evolution of O_2) and transferred to the chlorophyll unit. ATP is formed in this process also. In these ways green plants can generate high-potential reducing power (reduced triphosphopyridine nucleotide (TPNH)) and ATP from water and light energy. By an appropriate balance between the pathways, green plants can generate the exact amounts of ATP and TPNH needed for the conversion of CO_2 to cell materials. Purple bacteria perform cyclic phosphorylation but lack the ability to form high-potential reducing power and O_2 from water and light energy. To a first approximation they rely upon high-potential exogenous H-donors for whatever reducing power is needed in their growth.

Needless to say, the identity of enzymes and cofactors in the electron-transport pathways of Fig. 2, and also the details of the mechanism for oxidizing water, are in a state of controversy. Furthermore, a scheme proposing only cyclic phosphorylation in bacterial photosynthesis may be too simple. Succinate affords growth of purple bacteria but is not of sufficiently high potential to reduce pyridine nucleotide. In such a case one might introduce a pathway analogous to the one yielding TPNH as a product in green plants (*C* in Fig. 2), with succinate in place of H_2O and fumarate in place of O_2. Alternatively, it has been suggested (20, 42, 62) that ATP generates reduced pyridine nucleotide by driving an electron-transport system backward (against the potential gradient). Such a reaction has been demonstrated in mitochondria by Chance (20); the levels of ATP prevailing in plants (about 10^{-3} M) appear sufficient (85, 89) for this reaction. However, it is well established that in green plant chloroplasts the formation of TPNH is not a consequence of ATP formation (10, 72), and that in chromatophores of purple bacteria the succinate-linked reduction of diphosphopyridine nucleotide is actually inhibited by the simultaneous occurrence of photophosphorylation (42). It is still possible that another phenomenon, the photoinhibition of respiration in purple bacteria (21, 61, 95, 104), arises from the ability of ATP to drive oxidative electron transport backward.

From these considerations photosynthesis assumes the following character. Chlorophyll molecules, receiving light quanta, produce high-energy electrons. These are used to generate the energy (ATP) and the high-potential reducing power (reduced pyridine nucleotide) needed for the synthesis of cell materials from CO_2. In the photosynthetic bacteria ATP is often the only necessary photoproduct, the reducing power being supplied exogenously (as "H_2A") or not needed (when an external substrate is assimilated

directly in a reduced form). Our next consideration will be the manner in which chlorophyll molecules receive light quanta and deliver high-energy electrons to the enzyme machinery of photosynthesis.

III. Primary Photophysical Mechanism

A. Photosynthetic Unit; Models Taken from Solid-State Physics

There is now good theoretical (30, 31, 49), optical (17, 53), biochemical (90, 105), and morphological (13, 80) evidence that photosynthesis involves the cooperative action of many (e.g., about 200) chlorophyll molecules. The entire set of molecules cooperates in harvesting a single light quantum and storing its energy. This photosynthetic unit is a substructure in the green plant chloroplast; the units in purple bacteria might be the chromatophores. The chlorophyll in these structures probably exists as a two-dimensional quasi-crystal surrounded by lipid and protein, with little exposure to free water (13, 15, 38).

The idea of a large photosynthetic unit became more palatable with the recognition that excitation energy can be transferred with high efficiency among pigment molecules, provided that the molecules are no further apart than about 50 A (5, 26). Transfer of excitation energy occurs among pigment molecules in solution, but the photosynthetic unit is more than a bag of water containing molecules of chlorophyll-protein conjugate. It is a system having much structure and little water, and an understanding of its behavior should be sought, as Arnold and Sherwood have suggested (6), in the physics of the solid state rather than in the chemistry of solutions.

Indeed, it has been shown that photosynthetic tissues exhibit many solid-state properties suggesting that they are organic semiconductors.[5] Accordingly, the application of solid-state physics has engendered new hypotheses for the primary events in photosynthesis. (It was Katz (63) who first proposed semiconduction in a chlorophyll crystal as a mechanism in photosynthesis.) Before this theme is developed, a word of caution is appropriate. Solid-state theory and experiment have been harmonized through the study of inorganic semiconductors such as germanium crystals. Extension of the theory to such well defined organic semiconductors as anthracene crystals has already encountered severe difficulties. A further extension to photosynthetic tissues must therefore be made with great discretion. Germanium crystals may have capabilities not realized in an organic quasi-crystal of several hundred molecules, and the latter may have potentialities not suggested by the former.

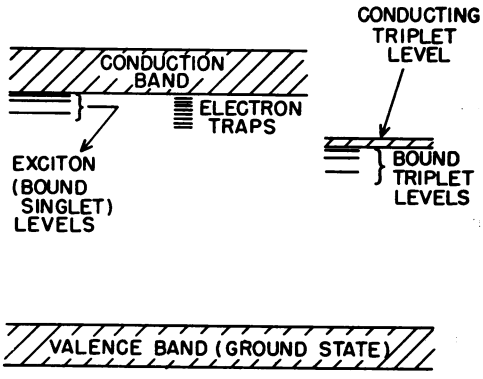

FIG. 3. *Electronic energy states in a semiconductor*

In a semiconductor (65), a photoexcited electron may exist in one of several distinct states, illustrated in Fig. 3. The conduction band represents a fusion of singlet excited states of the interacting molecules such that the orbit of an excited electron, and of the corresponding hole (electron vacancy), is delocalized over the entire crystal. The valence band is a similar fusion of ground state levels. A conduction electron (one in the conduction band) represents an ionized condition: the electron and the hole can move independently with no binding energy. The electron may become trapped spatially by losing a little energy at the site of a discontinuity (a flaw or a foreign molecule) in the crystal; the forces at the discontinuity must provide a metastable state (an electron trap level) for this to happen. Below the conduction band there are "exciton levels": excited states in which the electron is localized near the corresponding hole. A set of energy levels will exist, representing different binding energies (orbital radii) between the electron and the hole. An exciton of zero binding energy is the same as a conduction electron. Excitons can move through the crystal (5, 78); in this way excitation energy (but not

[5] The discovery of semiconductor phenomena in photosynthetic tissues was effected principally by Arnold. References are listed in Table 1, which is discussed in the next section.

free charge) is transferred. A weakly bound exciton can become dissociated by a thermal collision or an external electric field (giving a conducting electron and a conducting hole) or by encountering a trap that holds the electron. The trapped electron can re-enter the conduction band through a thermal excitation, or form a new exciton in an encounter with a hole. Finally it is possible (78) that triplet states, as well as singlets, can give rise to conduction bands and exciton levels. Holes, as well as electrons, can of course be trapped. In summary, the absorption of light in a semiconductor can lead to ionization (with migration of free electrons and holes), excitation (with migration of energy), and trapping of electrons and holes.

Owing to the uncertainties involved in applying solid-state considerations to photosynthetic tissues, the transition probabilities and lifetimes of the foregoing excited states cannot be predicted with great confidence. The theory imposes few restrictions favoring one mechanism or another for photosynthesis, and almost any point of view finds some experimental support.

The chief possibilities that are currently in vogue (78, 91) are outlined in Fig. 4. A chlorophyll unit is shown with a special acceptor site that abstracts a high-energy electron and channels it into the chemical machinery of photosynthesis. A low-energy electron is returned to the unit (or a hole is abstracted) at a special donor site. These sites may act as electron and hole traps in the unit. One possibility (*A*, Fig. 4) is that an exciton, generated by a light quantum, migrates to the acceptor site and dissociates there. The hole then finds its way to the donor site. Alternatively (*B*) the exciton dissociates at the donor site and the excited electron makes its way to the acceptor site. In both of these cases, the acceptor and donor sites may be so close to each other that very little migration of free charge in the chlorophyll unit is involved. Finally (*C*) the light quantum may place an electron in a conduction band, or may generate a loosely bound exciton that dissociates quickly into a conduction electron and a hole. The electron and the hole then migrate to the acceptor and donor sites, respectively. The migration of excitons or of electrons and holes can be channeled to the appropriate sites if the structure of the unit varies gradually, so as to produce suitable gradients in the electronic energy levels.

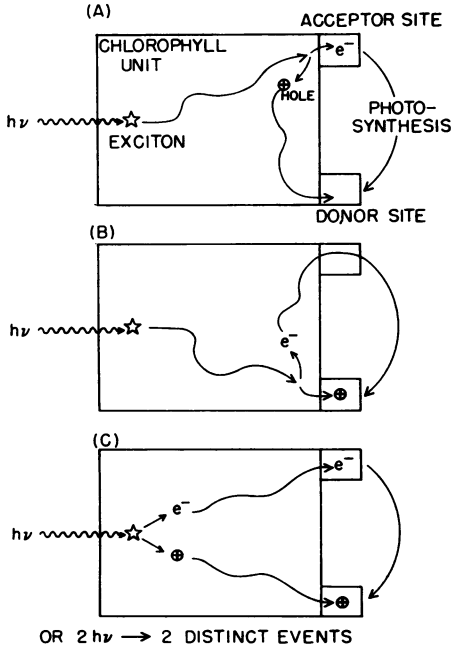

FIG. 4. *Some hypotheses for the primary photophysical process in photosynthesis.*

The appraisal of these models is fraught with such questions as these: "Is there a genuine conduction band, or are there zones of limited conduction embracing a few molecules?" "How numerous and how far apart are the acceptor and donor sites?" "Does photosynthesis require that two light quanta produce qualitatively different effects?" "Can both the electrons and the holes move?" The next to the last question will be discussed later. With regard to the last question, the weight of evidence is that holes conduct and electrons do not (50), or that electrons are quickly trapped, whereas holes remain free (74). But recent experiments using pure anthracene, in which artifacts (77) arising from contact with electrodes were eliminated, indicate that electrons and holes contribute equally to the conductivity of the crystal (64).

B. Experimental Development of Semiconductor Model

There is now abundant evidence that photosynthetic tissues, both dried and in their natural state, behave like organic semiconductors. Relevant phenomena are listed in Table 1, together with appropriate references. Photoconductivity,

photoinduced polarizability, delayed light emission, "glow curves," and the dependence of resistance on temperature are closely related phenomena that give direct evidence for ionization and electron trapping in a semiconductor. The kinetics and temperature dependence of electron spin resonance signals suggest that these are also manifestations of ionization and trapping. Suitable preparations (e.g., bacterial chromatophores) show light-induced optical transmission changes that are related to the foregoing phenomena.

Delayed light emission and reversible light-induced changes of conductivity, polarizability, and optical density have been studied comparatively in chromatophores and intact cells of *Rhodopseudomonas spheroides* (3). More recently the study of optical changes in these materials has been extended (R. K. Clayton, *unpublished data*). The light-induced changes in conductivity and polarizability give the most direct and unequivocal evidence for ionization, whereas the kinetics of these changes and the emission of delayed light reveal the extent of electron trapping. The information given by optical changes is less direct, but these changes can be studied conveniently in wet or dry preparations at temperatures from 1°K (supercooled liquid helium) to room temperature. In chromatophores, suspended in water or dried as films on glass, the optical changes reflect the same events (ionization and trapping) that produce the electrical changes and the delayed light emission. This is clear from the kinetics, the dependence on temperature and light intensity, and the effects of oxygen and of disrupting and drying the cells.

TABLE 1. *Solid-state phenomena in photosynthetic tissues*

Reversible Light-induced Changes:
a. Increased electric conductivity (4).
b. Increased electric polarizability (3).
c. Delayed light emission (2, 7, 15, 86, 87, 91–93).
d. Electron-spin-resonance signals correlated with a–c (15, 16, 19, 23, 24, 81, 91, 92).
e. Changes in absorption spectrum (dried preparations) correlated with a–d (3).

Other Phenomena:
f. "Glow curves": heat-induced luminescence after illumination (4, 6, 92).
g. Resistance vs. temperature: $\ln R = C + w/2k\ T$ (4, 6).

Figure 5 shows the absorption spectrum and the difference spectrum (illuminated minus dark) of a dry chromatophore film, prepared from a mutant of *R. spheroides* that lacks colored carotenoids. All the absorption bands above 300 mμ are those of bacteriochlorophyll. Wild-type *R. spheroides* shows, in addition, absorption bands and changes that are attributable to

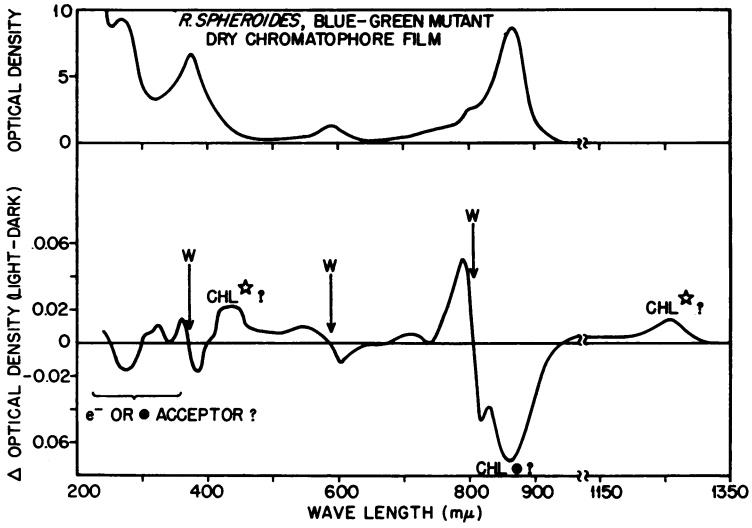

FIG. 5. *Absorption spectrum and difference spectrum (illuminated-dark) of a dry film of chromatophores from blue-green mutant Rhodopseudomonas spheroides.*

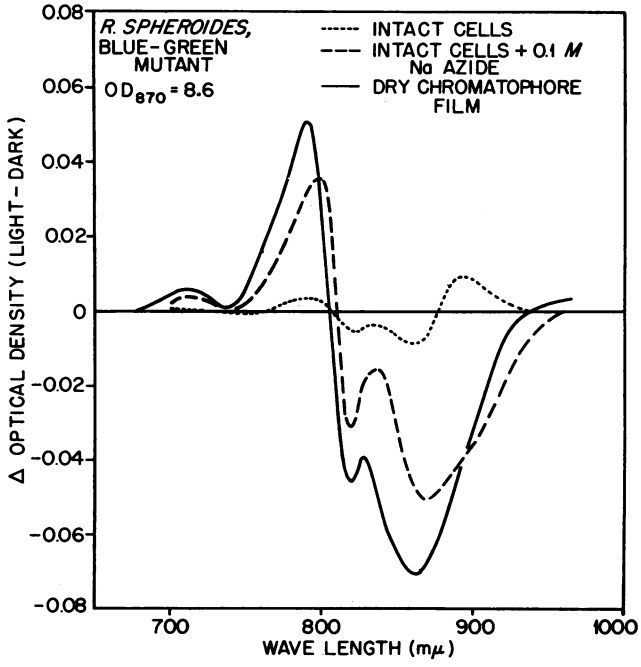

FIG. 6. *Difference spectra (illuminated-dark) of blue-green mutant Rhodopseudomonas spheroides: intact cells with and without 0.1 M sodium azide, and dry chromatophore film.*

carotenoid pigments, but carotenoids are not essential to bacterial photosynthesis.[6] The optical changes are freely reversible through hundreds of repetitions and the films are stable at room conditions for months. Chromatophores from *Rhodospirillum* and *Chromatium* species show essentially the same optical effects as do chromatophores from *R. spheroides*. The difference spectrum of Fig. 5 pertains to room temperature; it remains reversible and undiminished at temperatures down to 1 K. At any one temperature it shows the same kinetics, and the same dependence on exciting light intensity, at all wavelengths. The variation of kinetics and light-dependence with temperature shows that at low temperatures (1 K to about 150 K) the optical change is caused by ionization, and at higher temperatures (about 200 to 300 K) by ionization and trapping.

The difference spectrum of Fig. 5 may be dissected to reveal some consequences of photo-ionization in the chlorophyll unit. First, there are waves (*W*) around every absorption band of bacteriochlorophyll except the farthest infrared band. Rhodospirilla and chromatia also show waves around all but the longest wavelength band. The waves represent a shift of each band toward shorter wavelengths. The electric fields generated by electron-hole separation could cause such shifts by altering slightly the structure of the chlorophyll unit. The longest wavelength band would be exempt from the shift if it represents a deep (tightly bound) exciton level, strongly localized and uninfluenced by the crystal structure. The difference spectrum between 800 and 900 mμ resembles that of oxidized bacteriochlorophyll (54); this resemblance is seen also in wild-type *R. spheroides*, in rhodospirilla, and in chromatia. Bacteriochlorophyll molecules in the neighborhood of holes (Chl ⊕), being electron-deficient, may be regarded as oxidized. There are unidentified light-induced absorption bands (Chl*) at 420 to 450 mμ and at 1,255 mμ; these may represent excited-state transitions of bacteriochlorophyll. The difference spectrum in the region 240 to 350 mμ might possibly reflect reversible alterations of enzymes

[6] Mutants of *Rhodopseudomonas spheroides* have been isolated (22) that lack even the colorless polyene precursors of carotenoids, but show normal photosynthetic growth under anaerobic conditions.

or coenzymes attached to the unit, at electron-acceptor and donor sites.

With regard to these optical changes, chromatophores suspended in water behave the same as dried chromatophores. In living cells, in which the entire photosynthetic electron transport machinery in functioning, the optical changes associated with bacteriochlorophyll are much smaller. In such cells the main optical effect is one suggesting the oxidation of cytochromes. When intact cells are poisoned with hydroxylamine or azide their optical behavior becomes like that of dry chromatophores (Fig. 6). This transition parallels the decline in their capacity for photosynthesis. It appears that when the chemistry of photosynthesis is blocked, electrons and holes accumulate in the chlorophyll unit and manifestations of the primary photophysical process are revealed.

It can be argued (39) that the foregoing phenomena, in analogy with fluorescence, represent a derailment of normal photosynthesis, with excitation leading to ionization when the excitons cannot deliver their energy to the electron-transport systems. It is of interest, therefore, to determine the quantum efficiency of ionization. This problem is being approached by W. Arnold through an analysis of the kinetics and magnitude of the light-induced polarizability. The optical effect may also provide a quantum yield. The quantum efficiency for production of an electron-spin-resonance signal is high (16), but the signal need not arise exclusively from ionization.

In any case, a high quantum yield for ionization in washed or dried chromatophores does not prove that ionization is an essential step in photosynthesis. Furthermore, schemes based on exciton transfer (*A* and *B* in Fig. 4) predict a charge separation if the electrons or holes must move through an appreciable distance between acceptor and donor sites. The alternatives posed in Fig. 4 give different predictions concerning the steady-state abundance of isolated electrons and holes, and these predictions depend on whether or not the electron transport machinery is operating (compare living cells and dried preparations). Useful information could be gained, then, through a definite understanding of the optical effects produced by electrons and holes in a chlorophyll crystal. Electrons in a chlorophyll unit might produce a spectrum resembling that of reduced chlorophyll, and holes might cause an appearance of oxidized chlorophyll. The light-induced increased in absorption at 520 mμ, seen in green plants (79), is reminiscent of Krasnovsky's photoreduced chlorophyll (12, 68). Conversely, the light-induced changes in bacterial chromatophores (3, 27, 54, 66; also Fig. 5) suggest oxidized bacteriochlorophyll (54). Levitt (7) has pointed out a correspondence between the absorption bands of chlorophyll and the emission lines of neutral and single ionized magnesium (Mg^0 and Mg^+). One approach to the study of optical changes in chromatophores might be to add electron acceptors or donors, either to dry or wet preparations, in the hope of attacking light-induced electrons and holes. The meaning of the optical changes might then become clear, and a choice might be made between the mechanisms shown in Fig. 4.

C. The Emerson Effect: Red-Drop Phenomenon and Enhancement

One of the most active areas of research in photosynthesis in recent years has been the study of the Emerson effect (32): in green plants and algae the quantum efficiency of photosynthesis declines at wavelengths greater than about 680 mμ ("red drop"), but can be restored fully by admixture of shorter wavelength light ("enhancement"). A comprehensive review of this field is beyond the scope of this paper but some salient facts can be mentioned (1, 14, 15, 33–35, 38–40, 55–59, 66, 75, 78, 79).

The maximal rate of photosynthesis, under light saturation, is abnormally low with far-red light (79). Thus no amount of far-red light can reproduce the effect of shorter wave illumination. Furthermore, "extreme-red" light (740 mμ) inhibits the photosynthesis afforded by "far-red" light (690 mμ) (57). Enhancement can be observed not only under simultaneous illumination with far-red and other light, but also when the two qualities of light are applied sequentially with dark intervals of a few seconds (75).

Obviously a complete explanation of photosynthesis must allow for at least two distinct light reactions. One of these (with short wave light) is sufficient by itself; the other (with far-red light) can lead, at best, to an abnormally inefficient photosynthesis. A basis for two distinct light reactions has been sought in the existence of two distinct pigments (1, 66), or of chlorophyll

having two forms with different excited states (14, 26, 38, 56). The interaction between the two photoproducts can be imagined to occur at the primary photophysical level (59) or in subsequent chemical events (38).

One possibility is that far-red light can lead only to ATP formation via cyclic phosphorylation (paths *A* and *B* in Fig. 2), and that shorter-wave light is needed for the noncyclic process that generates reduced pyridine nucleotide as well as ATP. In green plants, cyclic phosphorylation (in contrast to oxygen evolution) seems to be exempt from the red-drop phenomenon (67). This suggestive experiment should be amplified; it may be that the Emerson effect is observable in chloroplasts when evolution of O_2 is the criterion of photosynthesis, but not when cyclic phosphorylation is the criterion.[7]

The photosynthetic bacteria do not evolve oxygen. They exhibit only cyclic phosphorylation, and for the most part do not make high potential reducing power at the expense of light energy. Moreover, the photosynthetic bacteria have not been observed to show the Emerson effect of red-drop and enhancement. It would be instructive to see whether these bacteria exhibit the Emerson effect when they are forced to utilize some light energy to generate high-potential reducing power (e.g., when they are growing at the expense of succinate). The Emerson effect should also be examined in H_2-adapted algae under two conditions: at low light intensity, such that they perform a "bacterial" photosynthesis, and at high light intensity, such that they revert to their normal "green plant" photosynthesis.

[7] Duysens, Amesz, and Kamp (27*a*) have now shown that in green algae a cytochrome is oxidized by far-red (680 mμ) light and reduced by shorter wave (562 mμ) light. Concurrently Losada, Whatley, and Arnon (70*a*) have shown that in chloroplasts one light reaction is responsible for oxygen evolution and another brings about phosphorylation and pyridine nucleotide reduction. Both groups offer the following explanation for the Emerson "red-drop and enhancement" phenomena: Far-red light, absorbed by chlorophyll a, promotes the oxidation of a cytochrome and the generation of high-potential reducing power. In this process electrons are abstracted from the cytochrome, raised to a higher potential, and given to an acceptor such as triphosphopyridine nucleotide. Phosphate is esterified concomitantly. Shorter wave light, absorbed by accessory pigments, promotes the transfer of electrons from H_2O to the cytochrome; in this reaction O_2 is evolved and reduced cytochrome is regenerated. For efficient photosynthesis both systems must be operating. Purple bacteria possess only the "far-red" system; the obligatory H-donor substrate is a source of electrons for the operation of this system.

IV. A Re-examination of Biochemical Unity

Thirty years ago photo- and chemoautotrophy were characterized as processes in which CO_2 was the sole source of cell carbon. Now the most distinctive feature in autotrophy is held to be the primary mechanism that provides energy and high-potential reducing power. It may indeed prove true that the only essential difference between photo- and chemoautotrophs resides in the nature of this primary mechanism (101). A corollary difference is that in chemoautotrophy, reducing and oxidizing equivalents are available independently, whereas in green plant photosynthesis the two are generated in equal amounts.

Throughout the evolution of current interpretations of photosynthesis and chemoautotrophy, discoveries in one field have led directly to corresponding discoveries in the other. The tricarboxylic acid cycle, uncovered in animal systems, was found in photosynthetic bacteria (28) when the idea of unity dictated that it should be. Ironically it was then found to be a major pathway only in the respiratory metabolism of these bacteria (29). Now the pentose cycle for CO_2 assimilation, unearthed in green plants (18), has been found in chemoautotrophs (88, 102). The discovery and interpretation of photophosphorylation was linked to the pre-existing model of oxidative phosphorylation (100), and the noncyclic phosphorylation of green plants may have its counterpart in hydrogenomonads (101). With investigation focused on the primary energy-yielding mechanisms, the submolecular processes invoked for one situation will surely find application in others. Thus, however the theories of autotrophy may change, the concept of unity will continue to exercise its powerful heuristic value.

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